IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Roger R. C. New

Serial No.: 10/553,169

Filed: April 15, 2004

For: UPTAKE OF MACROMOLECULES

DECLARATION

I, Roger R. C. New, do hereby declare and state as follows:

I am the inventor for US Serial No. 10/553,169 and have a thorough knowledge of the invention.

In the Office Action that issued on this application with a mail date of 5 August 2008 the Examiner suggests that it would have been obvious to add propyl gallate (PG) or butyl hydroxyl anisole (BHA) to a composition as described in the prior art document US 5,853,748. The compositions described in US 5,853,748 contain, *inter alia*, a bile acid or salt together with an agent with the ability to adjust the pH in the gut to a value of from 7.5 to 9. A preferred bile acid used in US 5,853,748 is chenodeoxycholate. A preferred pH adjuster used in US 5,853,748 is sodium bicarbonate.

I have conducted experiments to investigate whether or not it is actually possible to prepare a clear aqueous solution containing, along with chenodeoxycholate, both (i) sodium bicarbonate, and (ii) either PG or BHA.

First, I took a solution of 78.1mg chenodeoxycholate and 36.9mg PG in 1mL water and added 37.8mg sodium bicarbonate to it. The amounts of chenodeoxycholate and PG were chosen so as to replicate closely the 2:1 weight ratio that is used in the Examples of US Patent Application No. 10/553,169. The amount of sodium bicarbonate relative to the amount of chenodeoxycholate was chosen so as to replicate closely the 1:2 weight ratio that is used in Example 4 of US 5,853,748. An insoluble

mixture resulted. More specifically, a turbid dispersion was formed and even after incubation at 60°C it was still not possible to achieve a clear aqueous solution. Upon continued incubation for one hour at 37 °C, the mixture remained cloudy. Comparing this to an equivalent experiment wherein no sodium bicarbonate was added the difference was very marked throughout. In particular, the mixture without sodium bicarbonate formed a completely clear solution.

Second, I took a solution of chenodeoxycholate and sodium bicarbonate and added PG to it. The same amounts of the components were used as in the first experiment. A turbid dispersion was formed and even after incubation at 60 °C it was still not possible to achieve a clear aqueous solution.

Third, I conducted the first two experiments again but with the same weight of BHA in place of the PG. Similar results were obtained, i.e. a turbid dispersion was formed and no clear aqueous solutions were obtainable even after incubation at 60°C.

Signed

This 30th Day of October 2008.

THE
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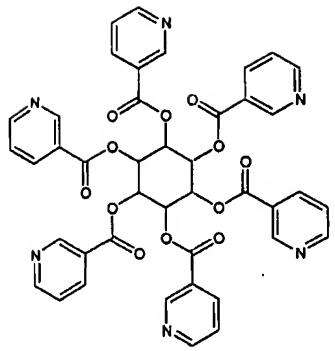
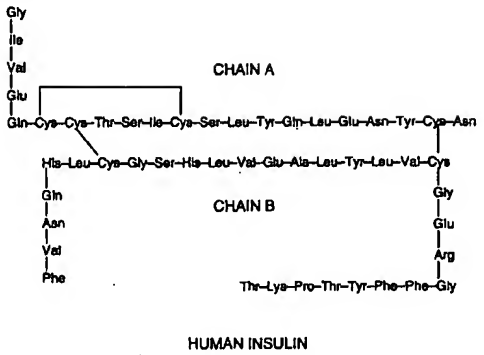


Crystals, mp 254.3-254.9°. Practically insol in water. Sol in dil acids.

THERAP CAT: Vasodilator (peripheral).

5003. Insulin. [9004-10-8]; [11061-68-0] (human). Polypeptide hormone produced by pancreatic beta cells that regulates carbohydrate homeostasis. Converted by proteolysis from the single chain proinsulin, *q.v.*, to the active dimer composed of 51 amino acid residues; mol wt ~6000. Regulates carbohydrate and lipid metabolism, and influences protein synthesis. Insulin was the first protein for which the chemical structure and mol wt were determined. Also the first commercial health care product produced by recombinant DNA technology. Because of its solubility at physiological pH, insulin is rapidly absorbed after subcutaneous injection. Various complexes with protamine and/or zinc have been prepd to improve drug delivery. In addition to biological source (human, porcine or bovine), insulin formulations for therapeutic use are classified according to onset and duration of action. Isoln: F. G. Banting, C. H. Best, *J. Lab. Clin. Med.* **7**, 251 (1921-22). Crystallization: Abel, *Proc. Nat. Acad. Sci. USA* **12**, 132 (1926). Purification and properties: J. Lens, *Biochim. Biophys. Acta* **2**, 76 (1948). Complete amino acid sequence of bovine insulin: F. Sanger, H. Tuppy, *Biochem. J.* **49**, 463, 481 (1951); F. Sanger, E. O. P. Thompson, *ibid.* **53**, 353, 366 (1953). Identification of 2 chain structure: A. P. Ryle *et al.*, *ibid.* **60**, 541 (1955). Review of structure determination: F. Sanger, *Science* **129**, 1340 (1959). Structure of human insulin: D. Nichol, L. F. Smith, *Nature* **187**, 483 (1960). Crystal structure: D. C. Hodgkin, *Verh. Schweiz. Naturforsch. Ges.* **150**, 93 (1970). Synthesis of human insulin: P. G. Katsoyannis *et al.*, *J. Am. Chem. Soc.* **88**, 164, 166 (1966); by the enzymatic modification of porcine insulin: M. A. Ruttenberg, *Science* **177**, 623 (1972). Review of synthetic insulins: P. G. Katsoyannis, *Recent Progr. Horm. Res.* **23**, 505-563 (1967). Synthesis of human insulin gene: H. M. Hsiung *et al.*, *Nucleic Acids Res.* **6**, 1371 (1979); **7**, 2199 (1979); **8**, 5753 (1980); S. A. Narang *et al.*, *Nucleic Acids Symp. Ser.* **7**, 377 (1980). Review of the development and production of human insulin by recombinant DNA technology: I. S. Johnson, *Science* **219**, 632-637 (1983). Molecular basis of insulin action: M. P. Czech, *Ann. Rev. Biochem.* **46**, 359 (1977). Review of biosynthesis: D. F. Steiner *et al.*, *Recent Progr. Horm. Res* **25**, 207-282 (1969). Review of the structure and function of the insulin receptor: J. Lee, P. F. Pilch, *Am. J. Physiol.* **266**, C319-C334 (1994). Symposium on the physiological regulation of insulin secretion and the pathogenesis of diabetes: *Diabetologica* **37**, Suppl. 2, S1-S187 (1994). Review of bioactivity, pharmacokinetics and therapeutic efficacy of human insulin: R. N. Brogden, R. C. Heel, *Drugs* **34**, 350-371 (1987). Review of insulin formulations and therapy: J. A. Galloway, R. E. Chance, *Horm. Metab. Res.* **26**, 591-598 (1994). History: M. Bliss, *The Discovery of Insulin* (Univ. Chicago Press, Chicago, 1982) 304 pp.

Crystals, hexagonal system, usually obtained as flat rhombohedra and contg 0.4% Zn. Readily sol in dil acids and alkalies. Isoelectric point 5.30 to 5.35.



HUMAN INSULIN

Bovine insulin. [11070-73-8] Hypurin.

Porcine insulin. [12584-58-6] Iletin II; Velosulin. Differs from human insulin by a single amino acid substitution.

Recombinant human insulin. Biosynthetic human insulin; insulin (prb); Huminsulin; Humulin; Humulina. Human insulin prepd by recombinant DNA technology. Clinical evaluation of inhaled intrapulmonary delivery in type 1 diabetes: J. S. Skyler *et al.*, *Lancet* **357**, 331 (2001); in type 2 diabetes: W. T. Cefalu *et al.*, *Ann. Int. Med.* **134**, 203 (2001).

Semi-synthetic human insulin. Insulin (emp); Biohulin; Novolin; Orgasuline. Human insulin prepd by enzymatic modification of porcine insulin.

Zinc insulin. [8049-62-5] Crystalline prepn of insulin containing 0.45-0.9% zinc. Formulated as suspensions in physiological saline; size of the particles determines the duration of action. Formulations are designated as prompt (or semilente), lente and extended (or ultralente).

Protamine zinc insulin. [9004-17-5] PZI insulin. Suspensions of insulin modified by the addition of zinc chloride and protamine sulfate. White or almost white suspension, pH 7.1-7.4. Onset of action occurs from 4-6 hrs after s.c. injection; duration of action is 36 hrs.

Isophane insulin. NPH insulin; neutral protein Hagedorn insulin. Crystallized prepn of protamine, zinc and insulin. Prepn: H. C. Hagedorn *et al.*, *J. Am. Med. Assoc.* **106**, 177 (1936). *Review:* P. Felig, *ibid.* **251**, 393-396 (1984). White suspensions of rod-shaped crystals ~30 nm in length, pH 7.1-7.4. Onset of action is 3-4 hrs following s.c. injection; duration of action is 18-28 hrs.

Insulin ^{131}I. Radio-iodinated insulin. Prepn: Burrows *et al.*, *J. Clin. Invest.* **36**, 393 (1957); Grodsky *et al.*, *Arch. Biochem. Biophys.* **81**, 264 (1959).

USE: Insulin ^{131}I used in the study of insulin binding factors from insulin resistant sera.

THERAP CAT: Antidiabetic.

5004. Insulinase. [9013-83-6] An enzyme that hydrolyzes insulin and is prepd from hog pancreas: Brink, Lewis, US 2957809 (1960 to Merck & Co.). May be obtained from commercial pancreatin or trypsin. Even the purified crystals contain large amounts of elastase. *Review:* Thomas, *Postgrad. Med. J. Suppl.* **49**, 940 (1973).

5005. Insulin Aspart. [116094-23-6] 28^{B}-L-Aspartic acid-insulin (human); AspB28-insulin (human); B28 asp-insulin; INA-X14; Novorapid. $C_{256}H_{381}N_{65}O_{79}S_6$; mol wt 5825.63. C 52.78%, H 6.59%, N 15.63%, O 21.70%, S 3.30%. Rapid-acting insulin analog produced by recombinant DNA technology. Identical to human insulin except for one amino acid substitution. Prepn: J. Brange *et al.*, *Nature* **333**, 679 (1988). Pharmacology and safety: V. Dall, *Arzneimittel-Forsch.* **49**, 463 (1999). Clinical pharmacokinetics and dynamics: S. R. Mudaliar *et al.*, *Diabetes Care* **22**, 1501 (1999). Clinical trial for postprandial glycemic control in type 1 diabetics: P. Raskin *et al.*, *ibid.* **23**, 583 (2000). Review of pharmacology

and clinical experience: K. L. Simpson, C. M. Spencer, *Drugs* **57**, 759-765 (1999).
THERAP CAT: Antidiabetic.

5006. Insulin Glargine. [160337-95-1] 21^{A}-Glycine-30^{Ba}-L-arginine-30^{Bb}-L-arginine-insulin (human); [Gly(A21),-Arg(B31),Arg(B32)]insulin (human); HOE-901; Lantus. $C_{267}H_{404}N_{72}O_{78}S_6$; mol wt 6062.99. C 52.89%, H 6.72%, N 16.63%, O 20.58%, S 3.17%. Long-acting analog of human insulin produced by recombinant DNA technology. Prepn: M. Dörschug, **DE 3837825**; *idem*, **US 5656722** (1990, 1997 both to Hoechst). Characterization of receptor interaction: L. Berti *et al., Horm. Metab. Res.* **30**, 123 (1998). Clinical pharmacodynamics: L. Heinemann *et al., Diabetes Care* **23**, 644 (2000); pharmacokinetics: D. R. Owens *et al., ibid.* 813. Clinical trial in type 1 diabetics: R. E. Ratner *et al., ibid.* 639. Review of clinical experience: P. S. Gillies *et al., Drugs* **59**, 253-260 (2000).
pI 6.7. Sol in acid pH. Insol at physiological pH.
THERAP CAT: Antidiabetic.

5007. Insulin-like Growth Factors. IGFs. Family of conserved peptide hormones structurally homologous with insulin. Two major circulating forms mediate the growth promoting effects of somatotropin, *q.v.* IGF-I regulates both prenatal and postnatal growth; IGF-II is a key factor in fetal development. IGFs are produced primarily in the liver under the regulation of growth hormone; also produced locally by most tissues. Transported in the serum by ***IGF binding proteins*** (or ***IGFBP***) that prolong the half-life and regulate the metabolic effects of IGFs. Discovered and termed ***sulphation factors*** because of their ability to stimulate the incorporation of sulfate by cartilage: W. D. Salmon, W. H. Daughaday, *J. Lab. Clin. Med.* **49**, 825 (1957). These peptides have also been referred to as ***NSILA-S***, or non-suppressible insulin-like acting substance. The designation ***somatomedin*** was proposed to connote the intermediary relationship to somatotropin: W. H. Daughaday *et al., Nature* **235**, 107 (1972). Discussion of nomenclature: *idem et al., J. Clin. Endocrinol. Metab.* **65**, 1075 (1987). Isoln, chemical characterization, biological properties of IGF-I and IGF-II: E. Rinderknecht, R. E. Humbel, *Proc. Nat. Acad. Sci. USA* **73**, 2365, 4379 (1976). Amino acid sequence of IGF-I: *eidem, J. Biol. Chem.* **253**, 2769 (1978); of IGF-II: *eidem, FEBS Letters* **89**, 283 (1978). Total synthesis of human IGF-I: C. H. Li *et al., Proc. Nat. Acad. Sci. USA* **80**, 2216 (1983); of human IGF-II: *eidem, Biochem. Biophys. Res. Commun.* **127**, 420 (1985). Review of molecular biology: W. H. Daughaday, P. Rotwein, *Endocrine Rev.* **10**, 68-91 (1989); of mechanism of action: A. Spagnoli, R. C. Rosenfeld, *Endocrinol. Metab. Clin. North Am.* **25**, 615-631 (1996). Review of IGF binding proteins: D. R. Clemmons, *Cytokine Growth Factor Rev.* **8**, 45-62 (1997). *Reviews:* C. E. H. Stewart, P. Rotwein, *Physiol. Rev.* **76**, 1005-1026 (1996); D. Le Roith, *N. Engl. J. Med.* **336**, 633-640 (1997).

Insulin-like Growth Factor I. [67763-96-6] IGF-I; somatomedin 1; somatomedin C; SM-C. Single chain, basic protein containing 70 amino acid residues. Review of physiology and potential therapeutic uses: E. R. Froesch *et al., Diabetes Metab. Rev.* **12**, 195-215 (1996).

Insulin-like Growth Factor II. [67763-97-7] IGF-II; multiplication-stimulating activity III-2; MSA III-2. Single chain, slightly acidic protein containing 66 or 67 amino acid residues depending on the species.

Mecasermin. [68562-41-4] Human insulin-like growth factor I. $C_{331}H_{512}N_{94}O_{101}S_7$; mol wt 7648.75. ***Myotrophin*** and ***Somason*** are recombinant products. Clinical trial in amyotrophic lateral sclerosis: D. J. Lange *et al., Neurology* **47**, Suppl. 2, S93 (1996).
THERAP CAT: In treatment of growth hormone insensitivity syndrome and insulin resistance.

5008. Insulin Lispro. [133107-64-9] 28^{B}-L-Lysine-29^{B}-L-prolineinsulin (human); [Lys(B28),Pro(B29)]-insulin (human); LY-275585; Humalog. $C_{257}H_{383}N_{65}O_{77}S_6$; mol wt 5807.66. C 53.15%, H 6.65%, N 15.68%, O 21.21%, S 3.31%. Rapid-acting insulin analog produced in *E. coli* by recombinant DNA technology. Identical to human insulin except for the transposition of proline and lysine at positions 28 and 29 on the B chain. Prepn: R. E. Chance *et al.*, **EP 383472**; *eidem*, **US 5514646** (1990, 1996 both to Lilly). Study of immunogenicity: C. M. Zwickl *et al., Arzneimittel-Forsch.* **45**, 524 (1995). General pharmacology: D. R. Helton *et al., ibid.* **46**, 91 (1996). Clinical comparison with regular human insulin: J. H. Anderson, Jr. *et al., Diabetes* **46**, 265 (1997). Review of development and pharmacokinetics: F. Holleman, J. B. L. Hoekstra, *N. Engl. J. Med.* **337**, 176-183 (1997); of clinical trials: V. A. Koivisto, *Ann. Med.* **30**, 260-266 (1998). Series of articles on pharmacology and clinical experience: *Acta Clin. Belg.* **54**, 233-254 (1999).
THERAP CAT: Antidiabetic.

5009. Integrins. Family of transmembrane glycoproteins involved in cellular adhesion and signal transduction. Name derived from their ability to "integrate" activities of the extracellular matrix (ECM) and the cytoskeleton. Integrins exhibit widespread evolutionary distribution: identified in mammals and other vertebrates, insects, and yeast; homologues have been identified in plants. At least 20 have been identified in mammals; composed of $\alpha\beta$ heterodimers selected from among 16 α and 8 β subunits. The α subunits vary in size from 120-200 kDa with ~1000 amino acid residues and usually consist of a heavy and light chain joined by a disulfide bond; β subunits generally range from 90-120 kDa with ~800 amino acids. Three major subfamilies have been characterized. Most integrins bind to the Arg-Gly-Asp (RGD) amino acid sequence found on components of the ECM, such as fibronectin, *q.v.* Others bind to cell membrane proteins, such as the intercellular cell adhesion molecules (ICAMs), or to soluble ligands, such as fibrinogen, *q.v.* Several recognize more than one ligand. Integrins anchor cells to the ECM or to adjacent cells, regulate cell spreading and motility, and transduce extracellular stimuli into a variety of intracellular signals. Implicated in a variety of physiological processes including embryological development, wound healing, immune functions, thrombosis, and metastasis. Identification of membrane glycoproteins involved in cell adhesion: D. E. Wylie *et al., J. Cell Biol.* **80**, 385 (1979). Identification of the transmembrane link between the ECM and the cytoskeleton: J. W. Tamkun *et al., Cell* **46**, 271 (1986). Description of integrin family: R. O. Hynes, *ibid.* **48**, 549 (1987); C. A. Buck, A. F. Horwitz, *Ann. Rev. Cell Biol.* **3**, 179-205 (1987). Role of RGD sequence in cell adhesion: E. Ruoslahti, M. D. Pierschbacher, *Science* **238**, 491 (1987). Review of integrin structure and ligand binding: A. Sonnenberg, *Curr. Top. Microbiol. Immunol.* **184**, 7-35 (1993); D. S. Tuckwell, M. J. Humphries, *Crit. Rev. Oncol. Hematol.* **15**, 149-171 (1993). Review of pharmacology: D. Cox *et al., Med. Res. Rev.* **14**, 195-228 (1994). Review of role in signal transduction: A. Richardson, J. T. Parsons, *BioEssays* **17**, 229-236 (1995); E. A. Clark, J. S. Brugge, *Science* **268**, 233-239 (1995).

β_1-Integrins. VLA antigens; VLA integrins; very late activation antigens. Widely distributed on various cell types. Share a common β_1 chain complexed with various α chains. Bind to ECM components such as fibronectin, collagen, laminin. Ca^{2+}/Mg^{2+}-dependent. Review: L. G. M. Baldini, L. M. Cro, *Leukemia Lymphoma* **12**, 197-203 (1994).

β_2-Integrins. Leukocyte integrins; Leu-Cam proteins; leukocyte adhesion molecules. Contain the β_2 chain; found on leukocytes. Bind to ICAMs, complement 3, and fibrinogen. Play an important role in regulating the immune system.

β_3-Integrins. Cytoadhesins. Contain the β_3 subunit; found on platelets, megakaryocytes and some melanoma cells. Ligands include fibrinogen, fibronectin, von Willebrand factor, vitronectin, and thrombospondin. Includes $\alpha_{IIb}\beta_3$, also known as ***platelet glycoprotein IIb/IIIa*** (***GPIIb-IIIa***), a fibrinogen receptor involved in platelet aggregation. Review: M. H. Ginsberg *et al., Thromb. Haemostasis* **70**, 87-93 (1993).

5010. Interferon. IFN. A family of species-specific vertebrate proteins that confer non-specific resistance to a broad range of viral infections, affect cell proliferation and modulate immune responses. Discovered by A. Isaacs and J. Lindenmann, *Proc. Roy. Soc.* **B147**, 258 (1957) while studying viral interference. Originally produced by the interaction of inactivated influenza virus with chick chorioallantoic membranes